Outbreak of Oropouche Virus in French Guiana

Mélanie Gaillet, Clara Pichard, Johana Restrepo, Anne Lavergne, Lucas Perez, Antoine Enfissi, Philippe Abboud, Yann Lambert, Laurence Ma, Marc Monot, Magalie Demar, Felix Djossou, Véronique Servas, Mathieu Nacher, Audrey Andrieu, Julie Prudhomme, Céline Michaud, Cyril Rousseau, Isabelle Jeanne, Jean-Bernard Duchemin, Loïc Epelboin,[1] Dominique Rousset[1]

Oropouche fever is a zoonotic dengue-like syndrome caused by Oropouche virus. In August–September 2020, dengue-like syndrome developed in 28 of 41 patients in a remote rainforest village in French Guiana. By PCR or microneutralization, 23 (82.1%) of 28 tested patients were positive for Oropouche virus, documenting its emergence in French Guiana.

French Guiana is an overseas territory of France in northern South America; 95% of the country is covered by Amazon rainforest. The remote village of Saül, deep in the rainforest, had 152 permanent inhabitants in 2017 (INSEE, https://www.insee.fr/fr/statistiques/4271842), but the actual population in 2020 was 95. The nurse of the health center keeps an updated count of inhabitants in the village, a number that was stable because of isolation during the coronavirus disease (COVID-19) pandemic. In August and September 2020, French Guiana was experiencing simultaneous COVID-19 and dengue outbreaks. Several inhabitants of Saül were treated for dengue-like symptoms, including fever and diffuse muscle pain, but rapid diagnostic testing for dengue was negative.

The Study

Saül houses 1 of 17 remote centers for prevention and care (RCPC) distributed throughout the inner territories of French Guiana (Figure 1). On August 11, 2020, a 55-year-old patient from Saül sought treatment with a dengue-like syndrome (DLS) including a marked meningeal component but tested negative for dengue. The patient was hospitalized on August 22 in Cayenne, the territorial capital. Bacteriologic, virologic, and parasitologic investigations were inconclusive. The Saül RCPC reported 15 additional patients with dengue-negative DLS during August 22–September 7. Consequently, an investigation was scheduled to begin in Saül on September 16. Sociodemographic data, clinical manifestations and evolution, and biological samples were systematically collected for each new case and, when possible, retrospectively for patients who sought treatment for DLS symptoms during August 11–September 16 (Appendix, https://wwwnc.cdc.gov/EID/article/27/10/20-4760-App1.pdf).

On September 22, because results of serologic testing for common locally circulating arboviruses were negative, we performed real-time PCR for Oropouche-like virus on all available samples collected ≤5 days after the onset of symptoms (*1*). We performed viral isolations on Vero cells from PCR-positive samples and sequenced 1 isolate. Later, we performed microneutralization tests to complete biologic investigations on late serum samples. We collected clinical, biological, and anamnestic data, including localization (Figure 2), from medical and laboratory records (Appendix).

As part of the entomologic investigation, over a 48-hour period during September 30–October 2, we captured potential vectors by using 11 BG-Sentinel traps (Biogents, https://biogents.com), 5 CDC light traps (BioQuip, https://www.bioquip.com), and 1 Woodstream Mosquito Magnet trap (https://www.woodstream.com). Vector control measures, mostly aerial insecticide spraying and larval treatment, were

Author affiliations: Cayenne Hospital Center, Cayenne, French Guiana (M. Gaillet, C. Pichard, L. Perez, P. Abboud, Y. Lambert, M. Demar, F. Djossou, V. Servas, M. Nacher, C. Michaud, L. Epelboin); Collectivité Territoriale de Guyane, Cayenne (J. Restrepo); Institut Pasteur de la Guyane, Cayenne (A. Lavergne, A. Enfissi, J.-B. Duchemin, D. Rousset); Institut Pasteur, Paris, France (L. Ma, M. Monot); Santé Publique France, Cellule Régionale Guyane, Cayenne (A. Andrieu, J. Prudhomme, C. Rousseau); Health Regional Agency of French Guiana, Cayenne (I. Jeanne)

DOI: https://doi.org/10.3201/eid2710.204760

[1]These authors contributed equally to the work.

only implemented 1 week later because of logistical constraints (lack of necessary aerial resources).

We obtained oral consent from patients to participate in the study and collected the biological samples as part of the care process. All data were collected on a standardized form and kept confidential to prevent disclosure of any personally identifiable information according to the requirements of the Commission Nationale de l'Informatique et des Libertés (https://www.cnil.fr).

During August 11–October 15, 2020, DLS was diagnosed in 41 (of 95 total) residents of Saül who sought treatment at an RCPC. Median age was 38 years (range 3–82 years, interquartile range 16–51 years) (Appendix Table 1); male-to-female ratio was 1.6:1 (Appendix Table 2). We tested blood samples from 28 patients; 23 were confirmed positive for Oropouche virus (OROV), 7 by PCR alone, 12 by microneutralization alone, and 4 by both. For the other 5 patients sampled, we were unable to confirm the diagnosis in the absence of a later sample to test for seroconversion. In addition, 17 residents, including 8 children, later reported having experienced DLS during the study period but did not visit the RPCP and therefore were not included in the study.

We obtained 5 viral isolates on Vero cells from PCR-positive serum samples; sequencing 1 of these isolates confirmed OROV infection. The attack rate in the village population was 43.2% (41/95); however, including residents with DLS symptoms who did not seek medical help would make the actual attack

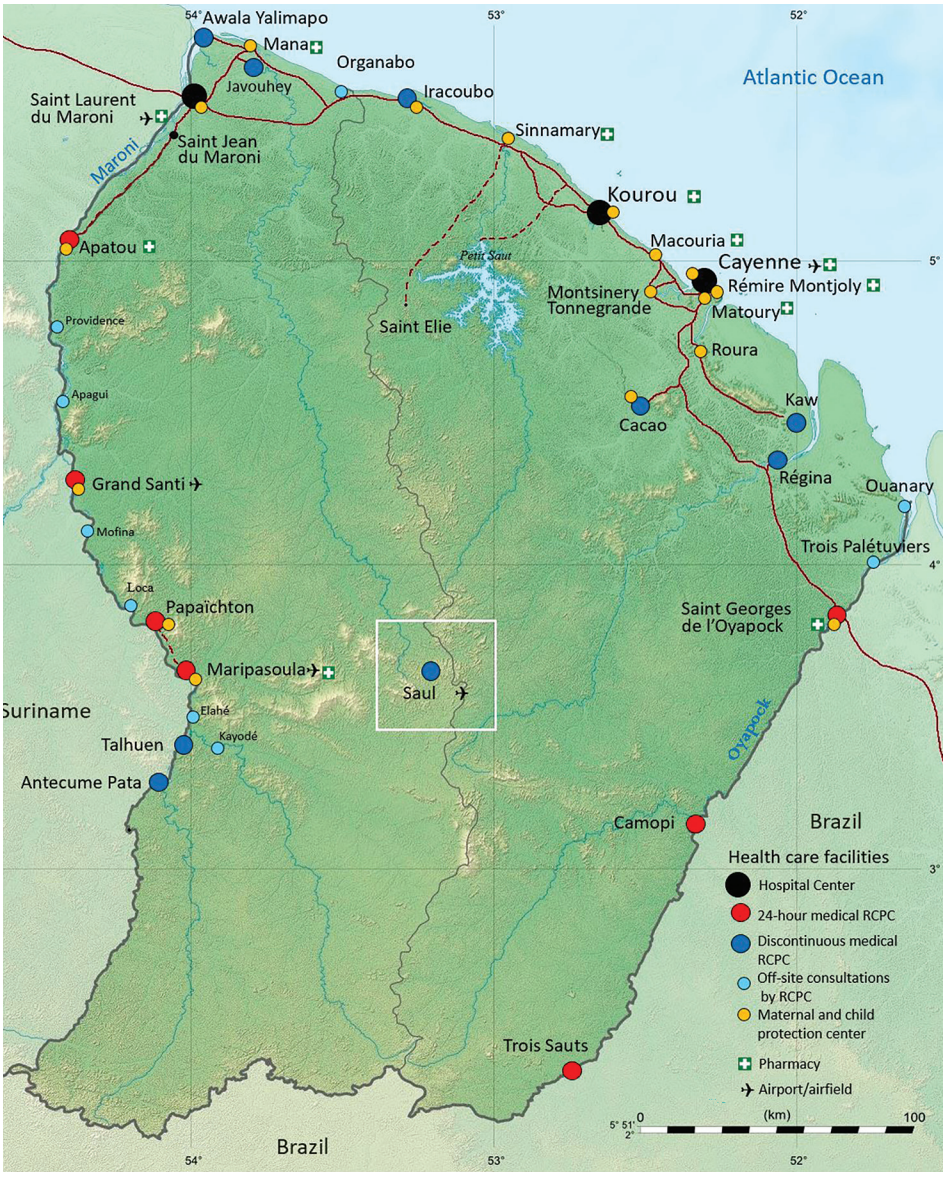


Figure 1. Locations of the town of Saül and 17 remote centers for prevention and care in French Guiana. Black circles: hospital centers; red circles: 24-hour remote centers for prevention and care; dark blue circles: remote centers for prevention and care (not 24-hour); light blue circles: off-site consultations with remote center for prevention and care; orange circles: maternal and child protection centers. Source: Dr. Elise Martin, Centre Hospitalier de Cayenne, French Guiana.

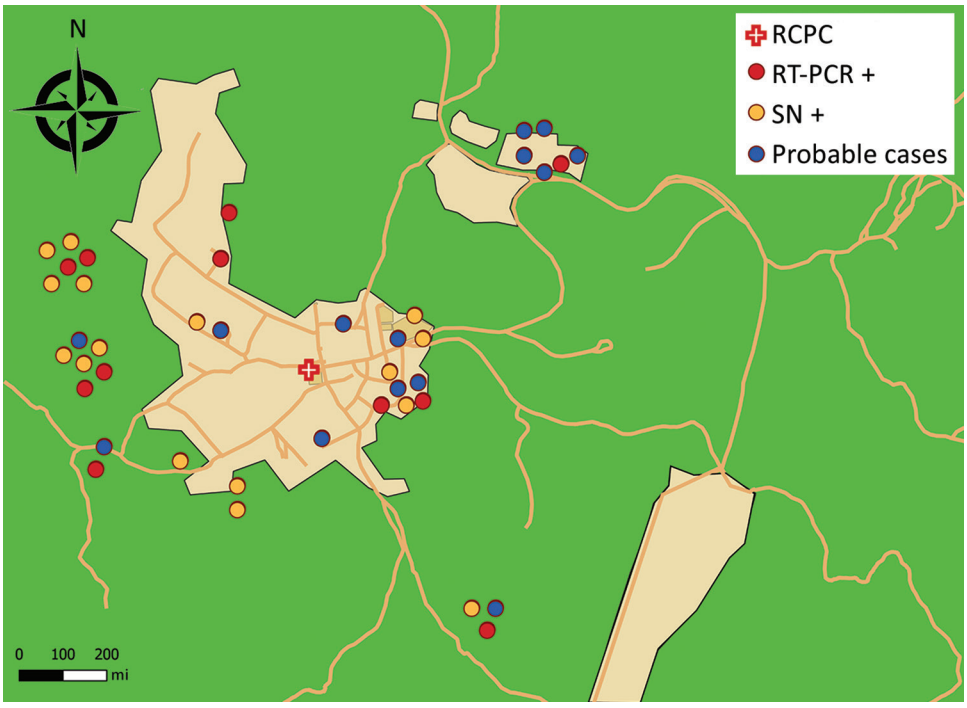


Figure 2. Spatial distribution of patient settlement around the town of Saül, French Guiana, and results of biologic testing for Oropouche virus by testing method. Geolocation is approximate to preserve patient anonymity. For probable cases (N = 18), samples were not taken. Green area, rainforest; light orange area, main districts of Saül; dark orange lines, forest trails. RCPC, remote centers for prevention and care; RT-PCR+, diagnosed with real-time PCR alone (N = 11); SN+, diagnosed with seroneutralization alone (N = 12).

rate 61.1% (58/95). Few patients had underlying conditions. Symptoms by order of frequency were fever, headache, myalgia, and asthenia (Appendix Table 2). The illness followed 3 successive phases: a 2–4-day acute phase, followed by a remission phase, then a rebound of symptoms ≈7–10 days after onset. Symptom intensity decreased by the end of the second week. Persistent tiredness was reported by 73.2% patients (30/41). Elevated CRP levels of up to 10 mg/L were observed in 5 (23%) of 22 patients and lymphopenia in 10 (42%) of 24. The outbreak peaked on September 16 (Figure 2), suggesting that transmission was slowing toward the end of September. The environmental vector control intervention was first applied on September 23 and then again the week of October 6–13. The disease affected all areas of the village of Saül; the index case-patient lived on the forest edge (Appendix Figure).

In total, during 36 nighttime trapping efforts, we collected 254 mosquitoes, 242 (95%) *Culex quinquefasciatus*, and 31 *Culicoides* (biting midges), only 1 of which was *C. paraensis*, which we trapped indoors with a BG trap. We captured the other midge specimens, mostly members of the *C. guttatus* group of subgenus *Hoffmania*, near a cocoa tree orchard close to the village.

Conclusions

Since the early 1960s, >30 OROV outbreaks have been reported, mainly in the northern states of Brazil (*2*,*3*), Peru, Ecuador (*4*), and Trinidad and Tobago, where OROV was first reported in 1955 (*5*). We report an outbreak of OROV fever in French Guiana. OROV is an arbovirus (genus *Orthobunyavirus*), transmitted through several vectors, including *C. paraensis* midges and *Cx. quinquefasciatus* mosquitoes in the urban cycle and *Aedes serratus* and *Coquillettidia venezuelensis* mosquitoes in the sylvatic cycle (*6*). Vertebrate hosts include sloths (*Bradypus tridactylus*) and monkeys (*Saguinus* spp., *Saimiri* spp., *Alouatta* spp.) (*7*). Because vectors and hosts both exist in French Guiana, the report of an OROV outbreak in this country was not unexpected.

OROV PCR is not routinely performed and serodiagnosis is not available in French Guiana; therefore, some individual cases of OROV infection not associated with an outbreak may have gone undetected. However, it is unlikely that many cases from past outbreaks went undiagnosed. Indeed, French Guiana is familiar with arbovirus outbreaks and has the resources to investigate them (*8*–*10*). Moreover, the high attack rate, homogeneous distribution of cases across the village, and different age groups affected in this outbreak imply the population had no immunity against OROV. The high attack rate could be explained by Saül's remoteness together with factors related to the COVID-19 pandemic. The village, which is accessible only by air, has been especially affected by the COVID-19 lockdown and subsequent

movement restrictions, which have isolated it even further. Also, a decrease in army presence in the surrounding forest has led to a substantial increase in illegal gold miners passing through from Brazil, which could have resulted in imported OROV. In addition, unmaintained forest trails around the village may have changed the vector density, but further entomologic studies are needed to test this hypothesis. We captured an abundance of potential vectors, especially *Cx. quinquefasciatus* mosquitoes, within the village itself. The low capture yield of local *Culicoides* spp. midges might have been linked to seasonal trends.

As described in the literature, clinical manifestations were moderately severe, and symptoms recurred among most of the patients studied (*11*). After the entomologic investigation, vector control measures were implemented in week 40. The near-exclusive presence in the village of *Cx. quinquefasciatus* mosquitoes among possible vectors suggests this species as the most plausible vector for this outbreak. However, because vectors were captured and sampled near the end of the outbreak, other potential vectors active earlier cannot be excluded. The presence of *Cx. quinquefasciatus* mosquitoes on the coast and in main cities of French Guiana and the geographic expansion of OROV in South America in recent years call for increased epidemiologic surveillance in this region (*12*).

Acknowledgments

We thank Séverine Timane Reillon, Frédéric Bouteille, Jean Yves Cattin, Fabien Rogalle, Fanny Gras, Sylvain Fradin, Romuald Carinci, Jean Issaly, Florence Jean Dit Gautier, Antonio Lopez, Mathilde Boutrou, Laure Lemée, David Moua, Laetitia Bremand, Bhety Labeau, Vincent Robert, Solène Wiedner-Papin for their involvement and willingness to help in the composition of this article.

About the Author

Dr. Gaillet is an infectious diseases specialist and epidemiologist. She created a mobile public health team in isolated communities in the most inaccessible villages of French Guiana, which intervenes on a wide range of public health issues, including the investigation of epidemics and increasing awareness of the prevention of coronavirus disease and many other topics.

References

1. Naveca FG, Nascimento VAD, Souza VC, Nunes BTD, Rodrigues DSG, Vasconcelos PFDC. Multiplexed reverse transcription real-time polymerase chain reaction for simultaneous detection of Mayaro, Oropouche, and Oropouche-like viruses. Mem Inst Oswaldo Cruz. 2017;112:510–3. https://doi.org/10.1590/0074-02760160062
2. Tilston-Lunel NL, Hughes J, Acrani GO, da Silva DE, Azevedo RS, Rodrigues SG, et al. Genetic analysis of members of the species Oropouche virus and identification of a novel M segment sequence. J Gen Virol. 2015;96:1636–50. https://doi.org/10.1099/vir.0.000108
3. Sakkas H, Bozidis P, Franks A, Papadopoulou C. Oropouche fever: a review. Viruses. 2018;10:10. https://doi.org/10.3390/v10040175
4. Wise EL, Pullan ST, Márquez S, Paz V, Mosquera JD, Zapata S, et al. Isolation of Oropouche virus from febrile patient, Ecuador. Emerg Infect Dis. 2018;24:935–7. https://doi.org/10.3201/eid2405.171569
5. Anderson CR, Spence L, Downs WG, Aitken TH. Oropouche virus: a new human disease agent from Trinidad, West Indies. Am J Trop Med Hyg. 1961;10:574–8. https://doi.org/10.4269/ajtmh.1961.10.574
6. Smith GC, Francy DB. Laboratory studies of a Brazilian strain of *Aedes albopictus* as a potential vector of Mayaro and Oropouche viruses. J Am Mosq Control Assoc. 1991;7:89–93.
7. Pinheiro FP, Travassos da Rosa AP, Travassos da Rosa JF, Ishak R, Freitas RB, Gomes ML, et al. Oropouche virus. I. A review of clinical, epidemiological, and ecological findings. Am J Trop Med Hyg. 1981;30:149–60. https://doi.org/10.4269/ajtmh.1981.30.149
8. Mutricy R, Djossou F, Matheus S, Lorenzi-Martinez E, De Laval F, Demar M, et al. Discriminating Tonate virus from dengue virus infection: a matched case-control study in French Guiana, 2003–2016. Am J Trop Med Hyg. 2020;102:195–201. https://doi.org/10.4269/ajtmh.19-0156
9. Epelboin L, Boullé C, Ouar-Epelboin S, Hanf M, Dussart P, Djossou F, et al. Discriminating malaria from dengue fever in endemic areas: clinical and biological criteria, prognostic score and utility of the C-reactive protein: a retrospective matched-pair study in French Guiana. PLoS Negl Trop Dis. 2013;7:e2420. https://doi.org/10.1371/journal.pntd.0002420
10. Bonifay T, Prince C, Neyra C, Demar M, Rousset D, Kallel H, et al.; Char Chik Working group. Atypical and severe manifestations of chikungunya virus infection in French Guiana: A hospital-based study. PLoS One. 2018;13:e0207406. https://doi.org/10.1371/journal.pone.0207406
11. Travassos da Rosa JF, de Souza WM, Pinheiro FP, Figueiredo ML, Cardoso JF, Acrani GO, et al. Oropouche virus: clinical, epidemiological, and molecular aspects of a neglected *Orthobunyavirus*. Am J Trop Med Hyg. 2017;96:1019–30.
12. Talaga S, Duchemin JB, Girod R, Dusfour I. The *Culex* mosquitoes (Diptera: Culicidae) of French Guiana: a comprehensive review with the description of three new species. J Med Entomol. 2021;58:182–221.

Address for correspondence: Loïc Epelboin, Unité des Maladies Infectieuses et Tropicales, Centre Hospitalier Andrée Rosemon et Université de la Guyane, Av des Flamboyants, F-97300, Cayenne, French Guiana, France; email: epelboincrh@hotmail.fr

Article DOI: https://doi.org/10.3201/eid2710.204760

Outbreak of Oropouche Virus in French Guiana

Appendix

Questionnaire

Patient n°:	Saul, the … /… / 2020	Approval: YES ☐ NO ☐
Last name:	First name:	Date of birth: _ _ / _ _ / _ _ _
Gender: M ☐ F ☐	Current job:	Phone number:

CLINICAL INFORMATION

Start Date of Signs: _ _ / _ _ / 2020

☐Fever> 38°5	☐Headache	☐Fatigue	☐Anorexia
☐Muscle pain	☐Arthralgia	☐Nausea/emesis	☐Chill
☐Cutaneous rash	☐ Retro-ocular pain	☐Diarrhea	☐Abdominal pain

Others:

ENTOURAGE INVESTIGATION

Number of people living under the same roof: _____	Location*:
Cases in the family environment: YES ☐ NO ☐	Name:
Number of people working together: _____	Location*:
Cases in professional environment: YES ☐ NO ☐	

*Patient Mapping

WAY OF LIVING

Sleeping under a mosquito net: YES ☐ NO ☐

Consumption of: ☐Game meat ☐Creek water ☐Others

Particular activities:

Animals:

_____

SAMPLING

YES ☐ NO ☐

Date of blood sampling: … / … / 2020 A D ... of the onset of symptoms

Last name: _____ First name: _____
Date of Birth: _____ D0 (Start Date of Signs): _ _ / _ _ / 2020

D0	D1	D2	D3	D4	D5	D6	D7	D8	D9
D10	D11	D12	D13	D14	D15	D16	D17	D18	D19
D20	D21	D22	D23	D24	D25	D26	D27	D28	D29

If symptoms marked "+" to be graduated according to their intensity: light "+" / moderate "++" / intense "+++".
If no symptoms leave the box empty.
Surround the symptoms present during the different "viremic phases".

Phase 1

FEVER	HEADACHE	FATIGUE	MUSCLE PAIN	ANOREXIA
DIARRHEA	NAUSEA/EMESIS	ABDOMINAL PAIN	CUTANEOUS RASH	ARTHRALGIA
RETRO-OCULAR PAIN	OTHERS, SPECIFY : …….			

Phase 2

FEVER	HEADACHE	FATIGUE	MUSCLE PAIN	ANOREXIA
DIARRHEA	NAUSEA/EMESIS	ABDOMINAL PAIN	CUTANEOUS RASH	ARTHRALGIA
RETRO-OCULAR PAIN	OTHERS, SPECIFY : …….			

Phase 3

FEVER	HEADACHE	FATIGUE	MUSCLE PAIN	ANOREXIA
DIARRHEA	NAUSEA/EMESIS	ABDOMINAL PAIN	CUTANEOUS RASH	ARTHRALGIA
RETRO-OCULAR PAIN	OTHERS, SPECIFY : …….			

Laboratory Methods

Sequencing

Viral isolations were performed on Vero cells from PCR positive samples, and 1 of the 5 isolates obtained was sequenced. Briefly RNA was extracted using Invitrogen™ TRIzol™ reagent according to manufacturer's recommendations. Total RNA was reverse transcribed into cDNA using the SuperScript® III Reverse Transcriptase (Invitrogen, Life Technologies, Inc.) and random hexamers (Roche, Mannheim, Germany) under the following thermal conditions: 65°C for 5 min, 25°C for 10 min, 50°C for 1 min and 75°C for 15 min. DNA samples were fragmented by Covaris M220 Focused-Ultrasonicator (Covaris Ltd, Brighton, UK) using microTUBE-15 to 350 bp. The TruSeq Nano libraries prep kit (Illumina) was used following the instructions of the kit manufacturer except 15 cycle of amplification due to the low amount of starting materials. Sequencing was carried out on Illumina MiSeq platform at a depth of 15 million reads total. Raw

reads were processed with an in-house bioinformatics pipeline for quality and variant calling (DOI: 10.21105/joss.00352) and assembled genome using spades (PMID: 32559359).

The sequences allowed the sequencing of the 3 segments of the Oropouche virus. Raw sequences were submitted to the Sequence Read Archive (SRA): SRA accession number SRR14711849.

Microneutralization

We performed microneutralization tests to complete biological investigations in order to confirm the diagnosis of Oropouche infection on late serum samples through the demonstration of a seroconversion. Briefly, we conducted the tests in serial 2-fold dilutions of heat inactivated sera starting at 1:10 mixed in equal volume with 100 tissue culture infectious dose 50 (TCID 50) of a French Guiana Oropouche strain (obtained after isolation on Vero cells culture from a Oropouche qRT-PCR positive sample of Saul). After incubation at 37 °C for 1 h, mixtures were transferred onto 96 well tissue culture plates containing subconfluent Vero cells. The neutralization titer was expressed as the reciprocal of the highest serum dilution at which infection is blocked. A serum was considered positive for titer >20.

Appendix Table 1. Biological, clinical, and anamnestic results (continuous variables) of confirmed and probable cases of Oropouche virus infection*

Category	No.	Median (25%–75% IQR)	Min.	Max.
Test results				
Hemoglobin, g/dL	25	13.2 (12.8–14.3)	11.7	15.2
WBC, G/L	25	5.8 (4.9–8.0)	3.1	11.6
PMN, G/L	25	3.8 (2.995–4.9)	1.9	8.7
Lymphocyte, G/L	25	1.4 (0.575–2.5)	0.2	3.3
Platelet count, G/L	25	237 (197–280)	67	399
SGPT, IU/L	24	21 (14–29)	10	76
SGOT, IU/L	17	25 (21–30)	15	49
CRP, mg/L	22	4.25 (1.0–9.3)	0.2	313.7
Age, y	41	38 (16–51)	3	82
Testing delay, d†	27	4 (1.5–9.5)	0	18

*CRP, C-reactive protein; Hb, hemoglobin; IQR, interquartile range; max, maximum; min, minimum; PMN, polymorphonuclear leukocyte; SGOT, serum glutamyl oxaloacetate transferase; SGPT, serum glutamic pyruvate transferase; WBC, white blood cell
†Time between onset of clinical signs and when first biological sample was taken

Appendix Table 2. Anamnestic and clinical results (categorical variables) of confirmed and probable cases of Oropouche virus infection

	Confirmed cases			Probable cases			Total population			
Category	N	Total	%	N	Total	%	N	Total	%	P
Age, y										
<18	5	23	22	6	18	33	11	41	27	0.49
>18	18	23	78	12	18	67	30	41	73	
Sex										
Male	14	23	61	9	18	50	23	41	56	0.54
Female	9	23	39	9	18	50	18	41	44	
Confirmed cases										
Total	23	23	100	NA	NA	NA	23	41	56	NA
By test method										
PCR alone	7	23	30	NA	NA	NA	7	28	25	
PCR and microneutralization	4	23	17	NA	NA	NA	4	28	14	
Microneutralization alone	12	23	52	NA	NA	NA	12	28	43	
Medical history										
Malaria	8	23	35	4	18	22	12	41	29	0.69
Leishmaniasis	2	23	9	1	18	6	3	41	7	1
Cardiologic	3	23	13	0	18	0	3	41	7	0.20
High blood pressure	0	23	0	2	18	11	2	41	5	0.18
Evolution										
Inpatient	3	23	13	0	18	0	3	41	7	NA
Outpatient	20	23	87	18	18	100	38	41	93	
Fever										
Yes	23	23	100	16	18	89	39	41	95	NA
No	0	23	0	2	18	11	2	41	5	
Headache										
Yes	21	23	91	17	18	94	38	41	93	0.62
No	2	23	9	1	18	6	3	41	7	
Myalgia										
Yes	16	23	70	13	16	81	29	39	74	0.47
No	7	23	30	3	16	19	10	39	26	
Fatigue										
Yes	18	23	78	11	15	73	29	38	76	1
No	5	23	22	4	15	27	9	38	24	
Loss of appetite										
Yes	8	22	36	9	15	60	17	37	46	0.19
No	14	22	64	6	15	40	20	37	54	
Abdominal pain										
Yes	4	21	19	1	14	7	5	35	14	0.62
No	17	21	81	13	14	93	30	35	86	
Diarrhea										
Yes	6	21	29	2	14	14	8	35	23	0.43
No	15	21	71	12	14	86	27	35	77	
Nausea/vomiting										
Yes	7	21	33	6	16	38	13	37	35	1
No	14	21	67	10	16	63	24	37	65	
Rash										
Yes	3	22	14	4	15	27	7	37	19	0.41
No	19	22	86	11	15	73	30	37	81	
Arthralgia										
Yes	3	23	13	1	16	6	4	39	10	0.63
No	20	23	87	15	16	94	35	39	90	
Chills										
Yes	5	7	71	4	6	67	9	13	69	1
No	2	7	29	2	6	33	4	13	31	
Retro-orbital pain										
Yes	12	23	52	7	15	47	19	38	50	1
No	11	23	48	8	15	53	19	38	50	

NA, not applicable

Appendix Table 3. Biological results (categorical variables) of confirmed and probable cases of Oropouche virus infection*

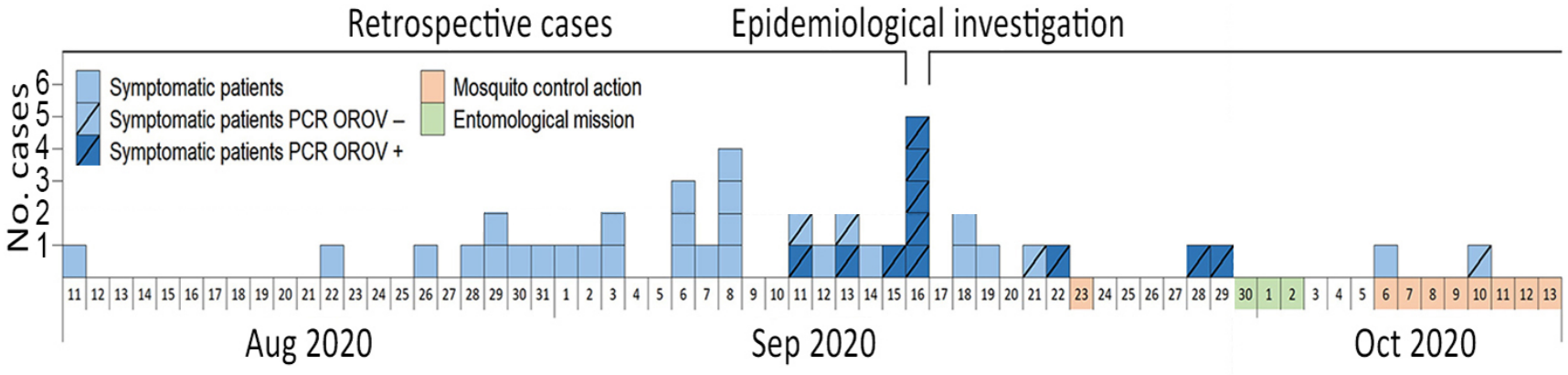
	Confirmed cases			Probable cases			Total		
Category	N	Total	%	N	Total	%	N	Total	%
Hemoglobin, g/dL									
<12	0	17	0	1	7	14	1	24	4
≥12	17	17	100	6	7	86	23	24	96
White blood cells, G/L									
<4	2	17	12	1	7	14	3	24	13
≥4	15	17	88	6	7	86	21	24	88
Polymorphonuclear leukocytes, G/L									
<1.4	0	17	0	0	7	0	0	24	0
≥1.4	17	17	100	7	7	100	24	24	100
Lymphocytes, G/L									
<1	9	17	53	1	7	14	10	24	42
≥1	8	17	47	6	7	86	14	24	58
Platelet count, G/L									
<150	2	17	12	0	7	0	2	24	8
≥150	15	17	88	7	7	100	22	24	92
Serum glutamic pyruvate transferase, IU/L									
≥40	2	16	13	2	7	29	4	23	17
<40	14	16	88	5	7	71	19	23	83
Serum glutamyl oxaloacétate transferase, IU/L									
≥40	0	13	0	1	4	25	1	17	6
<40	13	13	100	3	4	75	16	17	94
C-reactive protein, mg/L									
>10	4	16	25	1	6	17	5	22	23
≤10	12	16	75	5	6	83	17	22	77
C-reactive protein, mg/L									
>50	1	16	6	0	6	0	1	22	5
≤50	15	16	94	6	6	100	21	22	95
Toxoplasma gondii IgG									
Positive	8	13	62	2	4	50	10	17	59
Negative	5	13	38	2	4	50	7	17	41
Cytomegalovirus IgG									
Positive	9	10	90	6	6	100	15	16	94
Negative	1	10	10	0	6	0	1	16	6
Epstein-Barr virus viral capsid antigen IgG									
Positive	8	8	100	6	6	100	14	14	100
Negative	0	8	0	0	6	0	0	14	0
Dengue virus or nonstructural protein 1 PCR									
Positive	0	10	0	0	4	0	0	14	0
Negative	10	10	100	4	4	100	14	14	100
Dengue, Mayaro, chikungunya, or Saint Louis encephalitis virus IgM									
Positive	0	13	0	0	3	0	0	13	0
Negative	13	13	100	3	3	100	13	13	100
Yellow fever virus IgM									
Positive/borderline	4	13	31	1	3	33	4	13	31
Negative	9	13	69	2	3	64	9	13	69
Chikungunya IgG									
Positive	1	13	8	1	3	33	1	13	8
Negative	12	13	92	2	3	67	12	13	92
Zika virus IgG									
Positive/borderline	7	13	54	2	3	67	8	13	62
Negative	6	13	46	1	3	33	5	13	38
Leptospira IgM									
Borderline	1	10	10	1	5	20	2	15	13
Negative	9	10	90	4	5	80	13	15	87
Leptospira PCR									
Positive	0	6	0	0	3	0	0	9	0
Negative	6	6	100	3	3	100	9	9	100
Coxiella burnetii IgG									
Serological scar	4	11	36	0	5	0	4	16	25
Negative	7	11	64	5	5	100	12	16	75
Malaria smear									
Positive	0	2	0	0	1	0	0	3	0
Negative	2	2	100	1	1	100	3	3	100

*G, gigagrams

Appendix Table 4. Details of mosquito species captured during the entomological mission

Trap type	Trap/nights†	*Culex quinquefasciatus*	*Cx. bonneae*	*Cx. allostigma*	Other *Culex spp.*	*Anopheles spp.*	*Wyeomyia spp.*	*Uranotaenia spp.*	Total mosquitoes (average/trap)	*Culicoides spp.*	Phlebotomine
BG Sentinel	23	206	1	1	2	0	1	0	211 (9.2)	1*	1
CDC light traps	11	10	0	0	2	2	0	1	15 (1.4)	30	273
Mosquito Magnet	1	27	1	0	0	0	0	0	28 (28)	0	0
Total	35	243	2	1	4	2	1	1	254	31	274

**C. paraensis*
†Trap/nights, no. traps × no. nights



Appendix Figure. Patient settlement spatial distribution and OROV biological results. Geolocation is deliberately approximate to preserve anonymity. RCPC: remote centers for prevention and care; RT-PCR+: patients diagnosed by real-time PCR alone (N = 11); SN+: patients diagnosed by seroneutralization alone (N = 12); not sampled: probable case (N = 18); green: rainforest; light orange color: down town; dark orange lines: forest trails.